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Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives

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Abstract

A number of different marker-assisted selection (MAS) approaches do exist for the improvement of polygenic traits. Results of a marker-assisted backcross (MABC) selection experiment aimed at improving grain yield under drought conditions in tropical maize are presented and compared with alternative MAS strategies. The introgression of favourable alleles at five target regions involved in the expression of yield components and flowering traits increased grain yield and reduced the asynchrony between male and female flowering under water-limited conditions. Eighty-five per cent of the recurrent parent's genotype at non-target loci was recovered in only four generations of MABC by screening large segregating populations (2200 individuals) for three of the four generations. Selected MABC-derived BC₂F₃ families were crossed with two testers and evaluated under different water regimes. Mean grain yield of MABC-derived hybrids was consistently higher than that of control hybrids (crosses from the recurrent parent to the same two testers as the MABC-derived families) under severe water stress conditions. Under those conditions, the best five MABC-derived hybrids yielded, on average, at least 50% more than control hybrids. Under mild water stress, defined as resulting in <50% yield reduction, no difference was observed between MABC-derived hybrids and the control plants, thus confirming that the genetic regulation for drought tolerance is depen-

dent on stress intensity. MABC conversions involving several target regions are likely to result in partial rather than complete line conversion. Simulations were conducted to assess the utility of such partial conversions, i.e. containing favourable donor alleles at non-target regions, for subsequent phenotypic selection. The results clearly showed that selecting several genotypes (10–20) at each MABC cycle was most efficient. In the light of these results, alternative approaches to MABC are discussed, including recurrent selection, illustrated by an example of improving the adaptation of maize to low temperatures. Given the current approaches for MAS and the choices of marker technologies available now and potential for future developments, the use of MAS techniques in further improving grain yield under abiotic stresses in maize appears very promising.

Key words: Backcross, drought, marker-assisted selection, recurrent selection, *Zea mays*.

Introduction

Recent developments in plant molecular genetics have provided plant breeders with powerful tools to identify and select Mendelian components underlying both simple and complex agronomic traits (Ribaut and Hoisington, 1998; Dekkers and Hospital, 2002). Associations between markers and traits were first reported in maize by Stuber and

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Abbreviations: ASI, anthesis–silking interval; GEI, genotype by environment interaction; MABC, marker-assisted backcross; MARS, marker-assisted recurrent selection; MAS, marker-assisted selection; RFLP, restriction fragment length polymorphism; QTL, quantitative trait locus.

Moll (1972) using isozymes. The advent of abundant DNA-based molecular markers allowed the construction of genetic maps (Botstein *et al.*, 1980; Helentjaris *et al.*, 1986). Continuous progress in the areas of DNA technology, such as the discovery of the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), and genomics, have allowed the development of powerful genotyping systems, the construction of highly saturated maps (www.maizegdb.org; Lawrence *et al.*, 2004; Schaeffer *et al.*, 2006), and the establishment of countless marker–trait associations (Eathington, 2005). In addition to marker technologies, phenotyping technologies and statistical tools are critical to the ability to establish quality marker–trait associations (also called QTLs for quantitative trait loci). Over the past two decades, statistical methods to identify QTLs have evolved from rudimentary to very sophisticated. Significant milestones included the development of mixed model-based approaches (Jansen, 1993; Zeng, 1994; Jourjon *et al.*, 2005), models for the analysis of non-experimental populations (Thornsberry *et al.*, 2001), or approaches accounting for epistatic or genotype by environment interactions (GEIs; Jiang and Zeng, 1995; Vargas *et al.*, 2006). Phenotyping technologies and methodologies have also evolved. Spatial analysis (Gleeson, 1997), for instance, represents an improvement over alpha lattice or incomplete block analyses in terms of data quality. In addition, the benefits of spatial analysis are greater for stressed environments where GEI is usually large. Developments in large-scale low-cost technologies for the quantification of metabolites also represent significant improvements in phenotypic characterization. There is no doubt that the collection and use of very high quality phenotypic data are critical for the application of marker-assisted selection (MAS).

Despite numerous reports of QTLs in maize (www.maizegdb.org/qtl.php), including QTLs for adaptation to water-limited conditions, reviewed by Tuberosa *et al.* (2002), Ribaut *et al.* (2004), and Sawkins *et al.* (2006), little has been published on the implementation of MAS based on these QTLs in breeding programmes. Successful MAS applications have been reported for introgression breeding in maize, including introgressions of transgenes (Ragot *et al.*, 1995) and conversions involving simple (Ho *et al.*, 2002; Morris *et al.*, 2003) or complex traits (Bouchez *et al.*, 2002; Willcox *et al.*, 2002). Several marker-assisted recurrent selection (MARS) strategies have also been proposed and evaluated. When aimed at population improvement, MARS involves selecting individuals based on their marker genotypes and intermating them at random to produce the next generation (Hospital *et al.*, 1997; Knapp, 1998; Lande and Thompson, 1990; Moreau *et al.*, 1998). MARS can also be based on directed recombination between selected individuals of a segregating population, driven by an ideal genotype defined as a mosaic of favourable chromosomal segments from the parents of the segregating population (Stam, 1995; Van

Berloo and Stam, 1998, 2001; Charmet *et al.*, 1999). Although the use of MAS is increasing, it is not yet a common breeding practice, especially in the public sector. Besides cost, major limitations to the development of MAS might include the limited understanding of genetic phenomena such as gene networks, epistasis, and GEI.

Maize is the third most important food grain crop in the developing world. It is estimated that the demand for maize in developing countries will grow by 50%, from 558 million tons in 1995 to 837 million tons in 2020, outstripping the demand for wheat and rice. Much of this increased demand will be accommodated by increasing developing countries' domestic supply, which will require intensifying production on existing agricultural land (IFPRI, 2000). Since the majority of tropical maize is grown under rainfed conditions, the possibilities for alleviation of water stress are limited. Consequently, there is a strong need to assist farmers in the developing world by providing them with well-characterized cultivars that can not only withstand greater levels of drought but also perform well under optimum conditions. In an attempt to achieve this objective, CIMMYT has allocated significant resources over the past 15 years to improve grain yield under water-limited conditions in tropical maize. The research and discussions presented here are part of this effort.

The objective of this article is to review how MAS can contribute to maize breeding. Results of a marker-assisted backcross (MABC) experiment conducted at CIMMYT to improve grain yield under water-limited conditions in tropical maize are presented and discussed. Potential extensions of the MABC protocol aimed at opportunistically capturing favourable attributes from the donor parent are assessed through computer simulations. Finally, other approaches, including MARS, are discussed as possible alternatives to MABC and illustrated with an independent MARS experiment.

The drought adaptation MABC experiment in tropical maize

Plant material, phenotyping, and QTL detection

An MABC project was initiated in 1994 by crossing the drought-tolerant line Ac7643, the donor parent, and the drought-susceptible line CML247, the recurrent parent. CML247 is an elite tropical inbred line developed by CIMMYT and often used as a tester because of its outstanding combining ability and good yield *per se* under well-watered conditions. However, under water stress, CML247 displays a very large male–female flowering asynchrony (also known as ASI or anthesis–silking interval) and, as a consequence, is very susceptible to drought. A large ASI typically affects ovule pollination and therefore jeopardizes grain production (Bolaños and Edmeades, 1993). Ac7643 is tolerant to drought and has been previously

used as the drought-tolerant parent of a segregating population (Ac7643×c7729/TZSRW) used to identify QTLs for secondary traits and yield components under water-limited conditions (Ribaut *et al.*, 1996, 1997a). An F_{2:3} segregating population with 234 individuals was developed from the F₁ between Ac7643 and CML247. F₁ plants were also crossed to CML247 to generate a BC₁F₁ population.

All phenotypic evaluations were conducted during the dry winter season (November–April) in Tlaltizapan, Mexico (18°N, 940 m above sea level). Experimental designs consisted of alpha (0,1) lattices with two replications, similar to that described in Ribaut *et al.* (1996). Different water regimes were applied by restricting irrigation before and during flowering to obtain the desired drought-stress intensities. Irrigation was resumed at the end of the flowering stage, corresponding to the end of silk emergence, and maintained until harvest to allow grain filling. A well-watered regime was also conducted with irrigation applied every 12 d throughout the experiment.

Male (MFLW), and female flowering (FFLW) dates and yield component traits were collected. ASI was calculated as the difference between FFLW and MFLW for each plant (Ribaut *et al.*, 1996). Yield component traits including grain yield, number of ears per plant, 100 kernel fresh weight, and number of kernels per ear were recorded on a family basis. Means were adjusted using spatial analysis (Gleeson, 1997).

A genetic map was constructed from polymorphic loci using MAPMAKER (Lander *et al.*, 1987). Ten linkage groups were identified for a total map length of 1920 cM, and a mean marker density of 15.2 cM. The largest gap between two loci, on chromosome 9, was 46.2 cM. The order of the markers was found to be consistent with previously published maps.

QTL detection was performed using both simple interval mapping and two models of composite interval mapping (Zeng, 1994) as implemented in QTLCartographer (Basten *et al.*, 1994). Co-factors were selected by forward and backward regressions with in and out thresholds set at a *P*-value of 0.01. Outputs of the three models were compared to make final decisions on the presence or absence of QTLs. QTLs were considered to be significant when their LOD value was >3.0. Allelic effects, additivity, and dominance were obtained from QTLCartographer at each significant QTL.

Marker-assisted backcrossing

Target regions for MABC were identified primarily from QTLs for ASI. Significant QTLs detected in 1994 and 1996 from the segregating population Ac7643×Ac7729 (Ribaut *et al.*, 1996, 1997a) and in 1996 from Ac7643×CML247, where, in both cases, Ac7643 was the drought-tolerant parent, were used. Five QTLs for ASI, located on chromosomes 1, 2, 3, 8, and 10, were selected

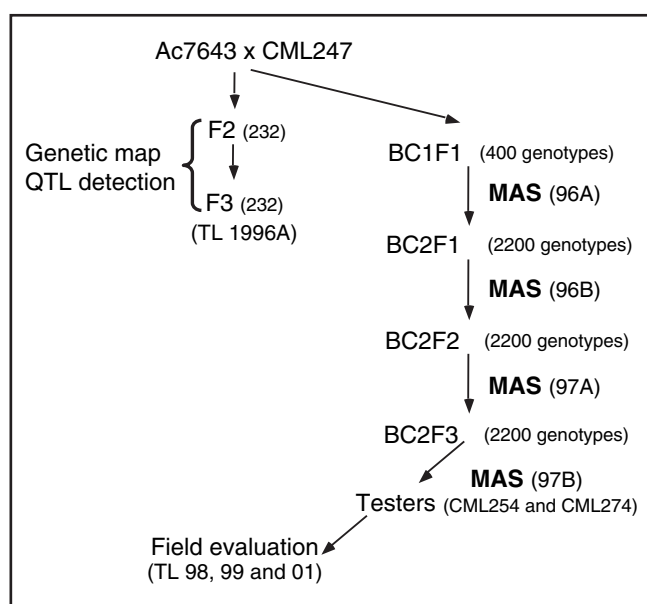


Fig. 1. General MABC scheme conducted at CIMMYT.

across these two segregating populations. These five QTLs either explained >5% of the phenotypic variance for ASI in at least one of the segregating populations, were detected in both populations, or co-localized with QTLs for yield components. Based on these observations, these five QTLs were used as target loci to transfer favourable alleles for yield production from Ac7643 into CML247 through MABC. A cluster of QTLs for flowering traits and yield components was identified on chromosome 3, where CML247 displayed the favourable alleles. Polymorphic molecular markers were used to ensure the fixation of the CML247 allele at an early stage of the line conversion in this region. Together, selected QTLs explained ~38% of the total phenotypic variance for ASI in the Ac7643×CML247 segregating population.

Details of the MABC experiment are shown in Fig. 1. Selected plants from the first two generations, BC₁F₁ and BC₂F₁, were advanced by backcrossing to the recurrent parent. Selected plants from the following two generations, BC₂F₂ and BC₂F₃, were advanced by self-pollinating. About 400 BC₁F₁ plants were genotyped for the first generation of marker-based selection. Subsequent generations involved the genotyping of ~2200 plants at each generation in an effort to increase the speed of the conversion process.

Marker-based selection was conducted in a stepwise manner on all four generations, from BC₁F₁ to BC₂F₃ (Fig. 1). Plants were first genotyped at three PCR-based markers which mapped within three of the target regions (on chromosomes 1, 2, and 10) and had been selected based on the quality of their polymorphisms and their combinability for multiplexed PCR amplifications (Ribaut *et al.*, 1997b). Plants displaying heterozygous genotypes

at these markers were selected before pollination to reduce the population size by 8-fold. Other genotypes were discarded. Then plants were genotyped at four pairs of restriction fragment length polymorphism (RFLP) markers flanking ASI QTLs located on chromosomes 1, 2, 8, and 10, plus one RFLP marker within the ASI QTL on chromosome 3. Plants displaying heterozygous genotypes at these markers were selected. Finally, for the second BC and the two self-pollinations, 60 polymorphic RFLP markers distributed throughout the genome were used to recover recurrent parent genotype at non-target regions. Plants with the highest proportion of CML247 alleles at these markers were selected. These last two selection steps involving RFLPs were conducted in the laboratory during grain filling and throughout harvest. At the end of each generation, ears from 10–15 individuals having Ac7643 alleles at target loci (within or flanking target regions) and the largest proportion of CML247 alleles at non-target loci (outside target regions) were selected to develop the next generation.

After the four MABC cycles, the 70 BC₂F₃ individuals presenting the closest allelic composition at target and non-target loci compared with the target genotype were crossed to two CIMMYT testers, CML254 and CML274. Ten individual CML247 plants were also crossed to the same two testers to produce 10 control hybrids. Eighty hybrids (70 MABC-derived and 10 controls) were thus evaluated for grain production in 1998, 1999, and 2001 under two water-stress regimes each year as well as a well-watered treatment in 1998 and 1999. From those 70 genotypes, 30 were selected based on their agronomic traits and yield performance. Doing so allowed capturing favourable alleles from Ac7643 outside the target loci and removing the genotypes presenting negative epistatic effects. Such selection was necessary as absolutely no phenotypic selection was applied during the MAS process.

Genetic composition of MABC-derived lines

Due to the large-scale selection that was conducted, the frequency of Ac7643 alleles at non-target loci for the best individuals was 13% after two backcross generations. These primarily flanked target regions due to linkage drag. This frequency decreased to 9% after the first self-pollination and 7% after the second self-pollination. The average frequency of donor genome at non-target loci across the 70 MABC-derived BC₂F₃ families was ~14%.

Simulation analysis of partial conversions

Selecting several genotypes at each cycle, as conducted in this MABC experiment, increases the probability of identifying at least one genotype that carries donor alleles at some non-target loci. However, increasing the number of selected genotypes per cycle delays the line conversion process due to an increase in the average of donor genome

contributions at non-target loci across selected genotypes. This trade-off was explored through simulations including screening at each cycle using a large population of 2000 genotypes and following the same model as described in Ribaut *et al.* (2002). The simulation results present the different average level of line conversion (left axis) expected across BC cycles considering that different numbers of genotypes had been selected at each cycle (5, 10, and 20) and the transfer of three or five QTL/target regions (15 cM each) (Fig. 2). The same figure indicates the expected percentage of the genome outside the selected regions at which the donor allele can be found in at least one selected genotype on both the carrying (with selected region) and the non-carrying (without selected region) chromosomes. Because the presence of donor alleles at non-target loci is random following Mendelian segregation on the non-carrying chromosome, but is affected by linkage drag at non-target loci linked to selected regions on the carrying chromosome, the probability of having a donor allele at non-target loci is always higher on non-carrying than on carrying chromosomes.

Independent of the level of conversion, the selection at each cycle of 20 genotypes compared with the selection of five genotypes increases the line conversion process by ~0.3 and 0.6 cycles for three and five QTL/target regions, respectively. Under the simulated conditions, 80–90% line conversion can be obtained after two (86% and 82% for five and 20 selected plants, respectively) and three (86% and 81% for five and 20 selected plants, respectively) BC cycles for the transfer of three and five target regions, respectively. After two BC cycles and the transfer of three regions, the donor allele would be expected to be found at 52, 77, and 98% of non-target loci on non-carrying chromosomes when selecting five, 10, and 20 genotypes per cycle, while those values decrease to 38, 64, and 90% on carrying chromosomes. For the transfer of five regions, after three BCs, donor alleles would be expected to be found at 45, 78, and 99% of non-target loci on non-carrying chromosomes for selecting five, 10, and 20 genotypes per cycle, while those values decrease to 25, 50, and 82% on carrying chromosomes. Those simulations clearly indicate that if the recovery of donor alleles at non-target loci is part of the selection strategy, then it is beneficial to select several genotypes at each MABC cycle. Increasing the number of selected genotypes from five to 20 almost doubles and triples the percentage of the genome where remaining Ac7643 alleles can be expected at non-target loci on non-carrying and carrying chromosomes, respectively. The gain from selecting several genotypes is larger for five compared with three regions selected for the MABC. As the self-pollination cycles will reduce the percentage presented in Fig. 3 following Mendelian segregation, it is important that elite donor alleles at non-target loci can be present in more than one genotype, another advantage for the selection of 20 genotypes at each cycle.

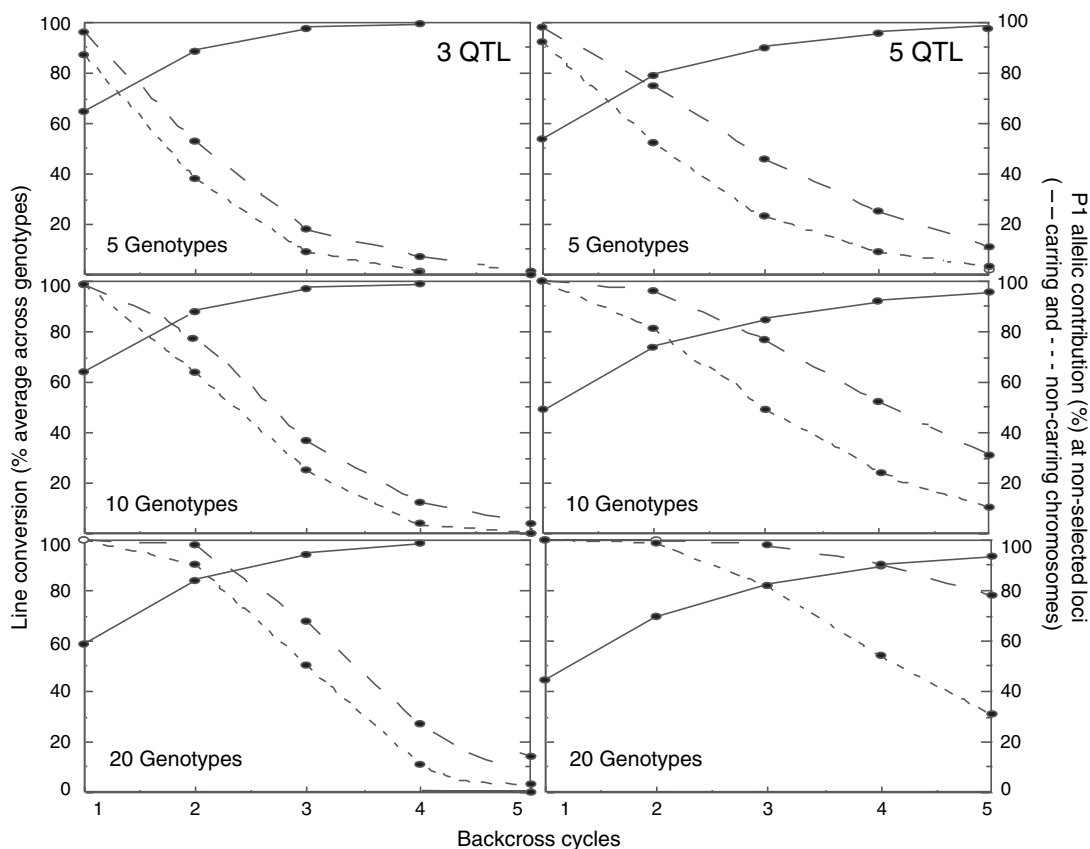


Fig. 2. Simulation results presented across BC cycles. The different average level of line conversion (left axis) expected considering the different number of selected genotypes (five, 10, and 20) and the transfer of three and five target regions (15 cM). Using the same parameters, simulations indicated the expected percentage of the genome outside the selected regions at which the donor allele on the carrying and non-carrying chromosome can be found in at least one selected genotype (right axis).

Field performance of MABC-derived lines

Among the 30 MABC-derived hybrids, a subset of five best hybrids performing consistently among the best genotypes for grain yield production across the drought trials was identified. Field performance (grain yield) of the three different groups of genotypes (controls, 30 MABC hybrids, and five best MABC hybrids) is presented in Fig. 3. From this figure, it is clear that performance of the three types of genotypes is significantly dependent on the level of stress. Under most severe stress conditions [severe stress (SS) and intermediate stress (IS) 1998], grain yield reduction (grain yield under stress versus well-watered for each group) for both IS and SS represented ~85% for the controls, 68% for the 30 MABC hybrids, and 43% for the five best MABC hybrids when crossed with CML254. With CML274, the yield reduction represented 86% for the control, 74% for the 30 MABC hybrids, and 68% for the five best MABC hybrids. Under those severe stress conditions, the five best MABC-derived hybrids yielded on average 2–3 times more than control hybrids, and the difference was larger for the hybrids obtained by using CML254 as tester compared with CML274. During 2001,

where the stress level was less severe compared with 1998, as demonstrated by the grain yield performance for the controls, the same tendency was observed but the difference among groups was reduced. The 1999 cycle was favourable for grain yield production due to the absence of high temperature stress during flowering and the presence of light rain, which reduced the level of stress. Those climatic conditions contributed to a very high yield under well-watered conditions, almost 73% and 39% more, compared with the yield performance under 1998 well-watered conditions for CML254 and CML 274, respectively. Although the grain yield reduction between stress and non-stress conditions was still ~45%, the trend indicated that the control-derived hybrids performed better compared with the 30 MABC- and five best MABC-derived hybrids with both testers.

These results demonstrate that genetic gains to improve grain production under severe stress conditions have been achieved through the use of molecular markers. Under those severe stress conditions (1998 and 2001), a subset of the five best derived-hybrids has been identified that performed significantly better in seven out of eight stress trials over those two years. The five BC₂F₃ lines used to

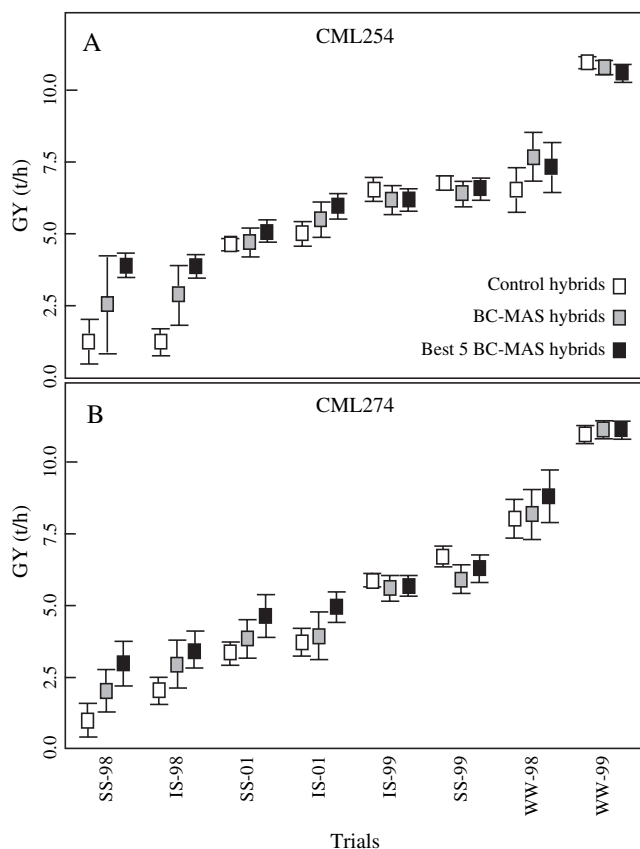


Fig. 3. Grain yield (GY) performance (t h^{-1}) of three types of hybrids obtained by crossing CML247 (controls) or selected MABC-derived BC2F3 families (30 MAS and best five MAS) with CML254 (A) and CML274 (B), evaluated under different water regimes across years. IS, intermediate stress; SS, severe stress; WW, well-watered.

derived the five best MABC hybrids were passed to CIMMYT's breeders to be evaluated and used in their respective target environments. When water-limited conditions did not reduce grain yield by more than 45% (1999), MABC-derived hybrids performed equally or less well compared with the controls, demonstrating that no genetic gain was obtained under those mild stress conditions. It is important to underline that no yield penalty was observed under well-watered conditions (1998 and 1999), neither for the 30 MABC hybrids, nor for the five best MABC hybrids compared with the control. Those results clearly indicate that different genetic mechanisms control the plant phenotype depending on the stress intensity, and that under moderate stress conditions grain yield is highly dependent on the yield potential of the cultivar and drought adaptation is less of an issue (Blum, 2006).

The MABC approach

Although several parameters influence what selection strategy should be followed, today the design of the most appropriate MABC strategy is relatively straightforward,

and genetic gain can be predicted through simulation (Frisch *et al.*, 2000). The transfer of five target regions using an optimal selectable population size of 50 genotypes (Ribaut *et al.*, 2002) can only be obtained by screening a large population, as was the case in this study for three cycles of selection (2200 genotypes). Due to the optimal selectable population size and because a target partial line conversion of 85% was chosen, the MABC presented here was completed in four cycles. Although the genetic gain has been evaluated in a single location, which had also been used for the QTL identification, thus reducing the potential impact of GEI, this experiment demonstrates the impact of MABC on grain yield improvement under water-limited conditions.

Selection against the donor genome in gene introgression is more effectively achieved by using molecular markers than by using conventional backcrossing (Tanksley *et al.*, 1989). Therefore, there is no doubt that MABC remains an efficient approach to transfer a single favourable allele (e.g. transgene) into different genetic backgrounds, or to improve a given genotype for a target trait. This latter approach is particularly important when breeding for human consumption, where the adoption of novel germplasm is challenging. The introgression of favourable alleles through MABC into farmer-favoured material, to improve it for a specific trait, is certainly one of the most suitable approaches for MAS in the South. Although the use of molecular markers to improve popular germplasm might have the largest short-term impact in developing countries, in the long term the transfer of favourable alleles from a donor to a recipient line is limited, as the output of such an approach can 'only' generate an improved version of an existing elite genotype. To overcome this limitation and take advantage of elite alleles coming from two or more parental lines, other MAS approaches can be considered, as described in the following cases.

MARS: a viable alternative?

MARS and MABC

Although phenotypic recurrent selection is well suited for improvement of complex traits in maize (Hallauer and Miranda, 1981), it has rarely been used due to the prohibitively long cycle length. With the advent of molecular markers and the dissection of traits into QTLs, an ideotype or ideal genotype can be defined as a mosaic of chromosomal segments (QTLs) carrying favourable alleles from various parents. In maize, performance is often measured as the simultaneous outcome of several traits. Consequently, ideal genotypes will often be complex genotypes. Stam (1995) provides an explanation why, in the case of bi-parental populations, these genotypes will never occur in any F_n population of realistic size. Similarly, because of the number of loci involved and the

relative contribution of the parents, the ideal genotype will generally not be attainable through MABC. MARS schemes, which involve several successive generations of crossing individuals based on their molecular marker genotypes, and the use of multitrait selection indices, would permit one to get close to or even achieve an ideal genotype (Peleman and Van Der Voort, 2003; Stam, 1995). The genetic gain achievable through MARS is probably higher than that achievable through MABC. For instance, when considering grain yield under both well-watered and water-limited conditions, MARS is likely to allow one to improve both traits, whereas MABC might restrict improvements to one of the two, as in the example shown above. More resources are likely to be needed for MARS than for MABC, although these may not be significantly larger if background markers are used to select for the recovery of the recurrent parent in the MABC approach.

MARS and phenotypic selection

Results from computer simulations have suggested that MARS was generally superior to phenotypic selection in accumulating favourable alleles in one individual (Van Berloo and Stam, 1998, 2001; Charmet *et al.*, 1999). The advantage of MARS over phenotypic selection was largest when the populations under selection were very diverse, as is often the case when breeding with tropical or subtropical material. Further simulation studies showed that the relative advantage of MARS over phenotypic selection decreases rapidly when the fraction of the total genotypic variance explained by the QTLs included in the selection index decreased (Van Berloo and Stam, 2001).

In terms of practical MARS applications, a number of published studies have shown contrasting results. Both Moreau *et al.* (2004) and Openshaw and Frascaroli (1997) showed little, if any, advantage of MARS over phenotypic selection, even though genetic gains obtained through MARS were reported. In contrast, several studies (Ragot *et al.*, 2000; Johnson, 2004; Crosbie *et al.*, 2006) reported successful large-scale applications of MARS in private maize breeding programmes. Ragot *et al.* (2000) identified QTLs in a bi-parental maize population and conducted MARS using a genetic index constructed from several traits including agronomic performance (grain yield, grain moisture at harvest) and adaptation to abiotic stress (early vigour under cold conditions). Genetic values were computed for the index as well as for individual traits for all generations of the MARS process, including the two parents of the population, demonstrating an increase of genetic values of individual traits in the desired direction when selecting individuals based on an index (Fig. 4). A report of an independent breeding programme by (Eathington, 2005) demonstrated that the rate of genetic gain achieved through MARS was about twice that from phenotypic selection. Several recent accounts of commer-

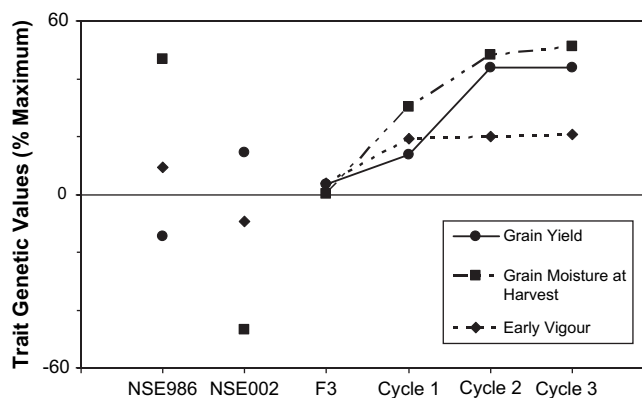


Fig. 4. Response to marker-assisted recurrent selection for agronomic performance and adaptation to abiotic stress. Genetic values are shown for all generations of the marker-assisted recurrent selection process, including the two parents of the population (NSE986 and NSE002) and the generation used for QTL mapping (F_3). Genetic values are expressed as percentages of maximum values. Genetic values are additive values and can therefore be positive or negative.

cial maize hybrids for which at least one of the parental lines had been derived through MARS also seem to suggest that MARS is being successfully implemented in private maize breeding programmes.

Multiallelic MARS

The most favourable alleles for traits of interest are likely to be spread across more than two lines, therefore requiring the assembly of alleles from many different sources in a single inbred line in order to achieve the greatest possible genetic gain. MARS can be used in such situations (Stam, 1995; Peleman and Van Der Voort, 2003). One obvious strategy would be the decomposition of the multiallelic situation into series of bi-allelic situations where the products of bi-allelic MARS schemes are brought together to assemble the final inbred lines. The use of MABC or partial backcrossing sequentially to pyramid favourable alleles from different sources might be a valuable alternative to the use of MARS if the number of target loci is low.

Yet, breeding with multiple sources of favourable alleles at once rather than conducting successive bi-allelic line development programmes could result in considerable time savings and therefore significantly improve the rate of genetic gain. The potential benefits justify investing in a thorough evaluation of multiparental MAS through simulation and practical research, and in a comparison of various realistic scenarios.

Other alternatives to MABC

The most critical challenge for MAS remains the ability to predict the phenotype of any given genotype in a segregating population based on the allelic constitution at target

loci. The complexity of gene networks and interactions among genetic elements or between genetic elements and the environment has often led to the discovery of fewer than expected stable significant QTLs across genetic pools and environments. This is a limitation which would strongly indicate that the probability of success of a MAS experiment can be increased where one detects QTLs in the target segregating population. However, the large number of QTLs characterized today cannot be ignored, nor can the large amounts of genomic sequence data available and the increasing amount of information produced through the '-omics' technologies allowing the identification of candidate genes and helping validating target regions of interest when co-localizing with QTLs. In this context, new approaches based on existing genetic information, without the need to map QTLs in any new cross—a very time-consuming and expensive step—becomes more and more appealing. One can envisage determining target regions identified through the construction of consensus genetic linkage maps from the compilation of genetic data generated through different approaches and collected over crosses and environments. The underlying rationale for this approach is that genes involved in a target trait are most likely to be located in the same position in the crop genome, and that phenotypic differences across germplasm are created by the nature/quality and interaction of the alleles at those genes. Therefore, one can postulate that if a genomic region has been identified from various segregating populations for a target trait, there is a high probability that the same region will be involved in the regulation of a target phenotype in new genetic backgrounds. This type of MAS based on the selection of target regions identified from previous studies is currently being conducted at CIMMYT for the improvement of drought tolerance in tropical maize. Preliminary results from this study are very promising as genetic gains have been observed for several target traits in a couple of new segregating populations.

All strategies discussed above have one thing in common. They pyramid favourable alleles using linked markers to target genes, or cluster of genes, at an early stage of recombination. However, there is no doubt that to predict allelic performance in a new segregating population or in multiparental design, informative markers at candidate genes will represent a major advantage in increasing the efficiency of the selection. Association studies have great potential for resolving which individual genes may be responsible for the expression of a target plant phenotype and have the power to evaluate and characterize a wide range of alleles (Buckler and Thornsberry, 2002; Flint-Garcia *et al.*, 2003a). Although common in human genetics in elucidating the genetic basis of a number of important human diseases (Lander and Schork, 1994; Risch and Merikangas, 1996), association approaches have only recently been applied to plant populations (Flint-Garcia *et al.*,

2003b). In maize, due to extensive recombination, only polymorphisms at no more than a few hundreds of bases to a locus responsible for the phenotypic effect are likely to be significantly associated with the trait in a randomly mating population (Thornsberry *et al.*, 2001). Thus informative polymorphisms characterized as gene haplotypes and associated with a contrasting phenotype can be converted into DNA markers for use in MAS experiments. Since the association approaches work with a wide range of germplasm, informative markers for most alleles affecting plant phenotype at a specific gene can be identified through association tests and used as a prediction tool in new and diverse genetic backgrounds. Combining genetic mapping with association studies has great potential for maize breeding.

Conclusions

There is no doubt that the use of molecular markers in public and private maize plant breeding programmes has increased significantly over the last few years, as demonstrated by the increasing number of published papers on this topic and the successful applications of MARS in the private sector. However, the potential of new approaches described herein and the present state-of-the-art technologies for the discovery and the use of markers also indicate that achieving the full potential of molecular genetics and genomics in contributing to maize improvement will necessitate further research on methodologies. Considering the seemingly exponential growth in the amount of genetic information being generated, there is no doubt that the major challenge for MAS will be in the integration of this diverse and disparate information and interpretation in a specific biological context to convert it into knowledge. For example, with the evolution of the current technology, one can state with some certainty that the haplotyping of several thousand genes of maize lines as a pre-breeding tool will soon become a reality. What may take longer and will be more challenging is to determine the biological value of the different alleles at those genes under target environmental conditions. This challenge is even greater for target environments exhibiting a large GEI such as water-limited environments. Modern maize breeding for complex traits, a combination of molecular and phenotypic selection, should become routine in breeding programmes, but success will largely be dependent on the accuracy of plant phenotyping and the capacity to determine the GEI; two major components that affect the prediction of the allelic value on the plant phenotype in new populations.

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